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Analysis of Physiological Data from the International Clone Trial (ICT)

A.J. Daymond and P. Hadley

School of Biological Sciences, The University of Reading, Whiteknights, Reading. RG6 6AS, UK

Abstract

A range of physiological parameters (canopy light transmission, canopy shape, leaf size, flowering and flushing intensity) were measured from the International Clone Trial, typically over the course of two years. Data were collected from six locations, these being: Brazil, Ecuador, Trinidad, Venezuela, Côte d'Ivoire and Ghana. Canopy shape varied significantly between clones, although it showed little variation between locations. Genotypic variation in leaf size was differentially affected by the growth location; such differences appeared to underlie a genotype by environment interaction in relation to canopy light transmission. Flushing data were recorded at monthly intervals over the course of a year. Within each location, a significant interaction was observed between genotype and time of year, suggesting that some genotypes respond to a greater extent than others to environmental stimuli. A similar interaction was observed for flowering data, where significant correlations were found between flowering intensity and temperature in Brazil and flowering intensity and rainfall in Côte d'Ivoire. The results demonstrate the need for local evaluation of cocoa clones and also suggest that the management practices for particular planting material may need to be fine-tuned to the location in which they are cultivated.

Introduction

Various studies have demonstrated genotypic variability in cocoa for a range of physiological traits. These include photosynthetic parameters (Baligar *et al*. 2008; Daymond *et al*. 2010) and canopy traits (Yapp and Hadley 1994; Daymond and Hadley 2002). From controlled environment studies on a limited range of cocoa germplasm evidence has been found of differential effects of temperature on growth, photosynthetic efficiency, canopy development and fruit development of contrasting cocoa genotypes (Daymond and Hadley 2004 and 2008). However, little work has been conducted on the stability of physiological traits in cocoa under field conditions. The International Clone Trial established in the CFC/ICCO/Bioversity project thus provides an opportunity to examine physiological traits in cocoa across a range of geographical locations.

The specific aims were: to estimate the extent of variability in physiological traits across the clones used in the International Clone Trial and to assess the stability of such traits within and across sites hence determining whether some clones are more susceptible to environmental stimuli than others.

Methodology

The design of the International Clone Trial is described by Eskes (2000). The trial has six blocks and eight replicate trees per block. A standardised set of working procedures were distributed to participating institutes early in the project. Data were collected from the International Clone Trial at six locations, these being: CEPEC/CEPLAC (Brazil), INIAP (Ecuador), MALMR (Trinidad), INIA (Venezuela), CRIG (Ghana) and CNRA (Côte d'Ivoire). The data collected at each site is summarised in Table 1.

Canopy Characteristics
Canopy shape was determined using an index of 1-5, where 1 represents a horizontal spreading canopy shape and 5 a vertical erect canopy shape. For light transmission, a 0-5

scale was used corresponding to 0, 5, 10, 20, 40 and > 50% light transmission. These measurements were made at the end of the rainy season, before pruning in 2006/7 (2000-2002 for Ecuador). Leaf size was measured in terms of leaf length and width of all leaves on two flushes on two trees in all of the six blocks.

Flowering and Flushing Patterns
Flushing and flowering intensity were recorded on a monthly basis in five locations (Brazil, Côte d'Ivoire, Ecuador, Trinidad and Venezuela) over the course of 2007/8. For these data sets indexes were used as summarised in Table 2.

Table 1: Summary of data collected at each site where the CFC International Clone Trial is located. Data refer to the number of times each parameter was measured.

Institute/ Country	Flowering intensity	Flushing intensity	Leaves per flush	Leaf size	Canopy shape	Light interception
CEPEC, Brazil	13	13	1	2	1	1
INIAP, Ecuador	10	10	-	-	1	-
MALMR, Trinidad	14	16	4	2	1	2
INIA, Venezuela	12	12	-	2	2	2
CRIG, Ghana	1	1	-	-	1	1
CNRA, Côte d'Ivoire	12	12	-	1	1	1

Table 2: Indices used for recording flushing and flowering intensity

Index score	Flushing	Flowering
0	0% flushing	0 flowers
1	20% flushing	1-5 flowers
2	40% flushing	6-15 flowers
3	60% flushing	16-50 flowers
4	80% flushing	51-150 flowers
5	100% flushing	150+ flowers

Statistical Analysis
Parameters were initially analysed within a given country for the effect of clone and, where applicable, the effect of time of year using analysis of variance (ANOVA). Where parameters were measured in more than one country, two-way analysis of variance was used to analyse the effect of clone and country.

Results

Canopy Shape
Canopy shape was recorded by five participating countries (Brazil, Ecuador, Ghana, Côte d'Ivoire, Trinidad and Venezuela). In an analysis of 11 clones common to the five sites, significant differences were found between clones ($P<0.001$) with SCA 6 having the most spreading and SPEC 54/1 the most erect canopy (Figure 1). No significant differences were observed between sites.

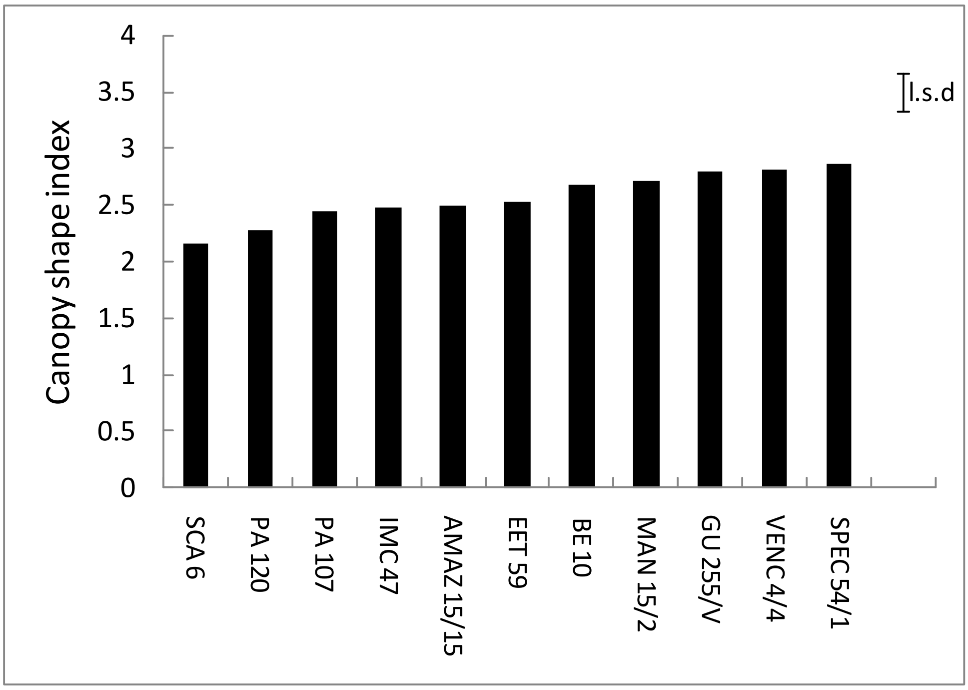

Figure 1: Canopy shape of 11 clones within the International Clone Trial (1 represents a spreading canopy and 5 an erect canopy). Values are the means across five different sites.

Light transmission

Light transmission was measured in four countries (Brazil, Ghana, Côte d'Ivoire and Trinidad). An analysis of ten clones common to the four sites demonstrated significant differences in light transmission between clones and across countries ($P<0.01$ in both cases). Furthermore, there was a significant clone x country interaction ($P<0.05$); for example, the clone IMC 47 exhibited a reasonably constant level of light transmission in all four sites, whereas SCA 6 exhibited a higher level of light transmission in Trinidad than at the other three sites.

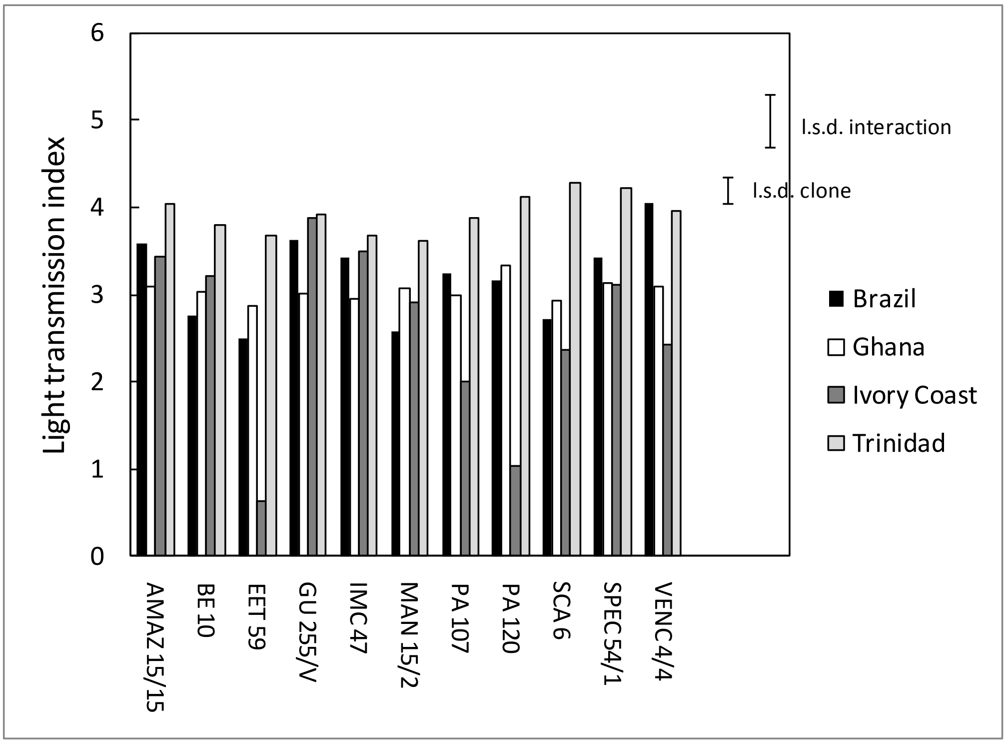

Figure 2: Light transmission of 11 clones within the International Clone Trial at four locations. (1 represents a low level of light transmission; 5 a high level).

Number of leaves per flush was measured in Trinidad and in Brazil. The number of leaves varied significantly between clones (P<0.001) from an average of 4.1 for PA 107 to 5.2 to MXC 67. Whilst leaf number was consistently higher in Brazil (P<0.001) the magnitude of this difference was greater for some clones than others; hence the clone x country interaction was significant (P<0.05; Figure 3).

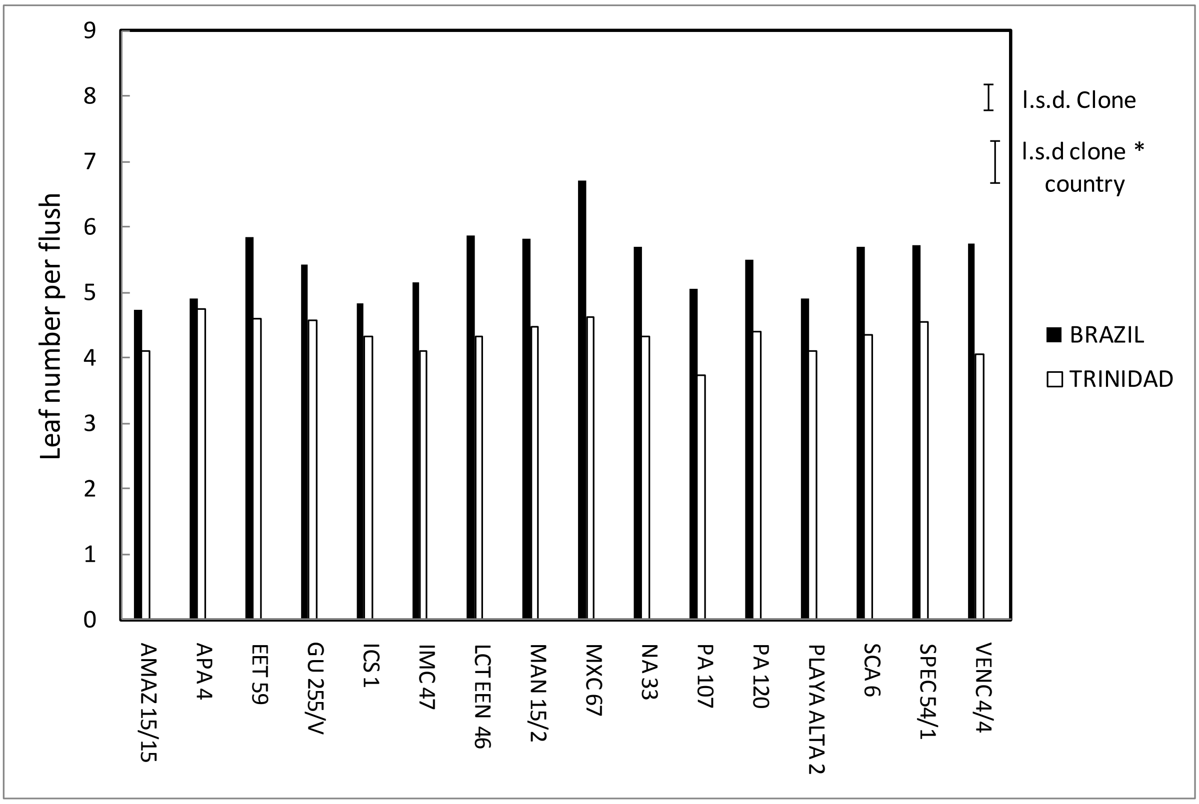

Figure 3: Leaf number per flush of 16 clones in the International Clone Trial in Brazil and Trinidad.

Leaf size

Leaf size (recorded as length and width) was measured at three locations: Brazil, Côte d'Ivoire and Trinidad; in Brazil and Trinidad leaves of different ages were distinguished. A comparison of leaf length of "leaf 1" (the youngest fully hardened leaf of a given flush) of 14 clones grown in Brazil and Trinidad is presented in Figure 4. Significant differences were observed between clones (P<0.001) the average length varying from 19.2 cm for SCA 6 to 31.8 cm for PA 120. On average, leaves were 1.3 cm longer in Trinidad compared to Brazil (P<0.01). However, the magnitude of the difference between sites was considerably different between clones; hence there was a significant interaction between clone and location (P<0.001; Figure 4).

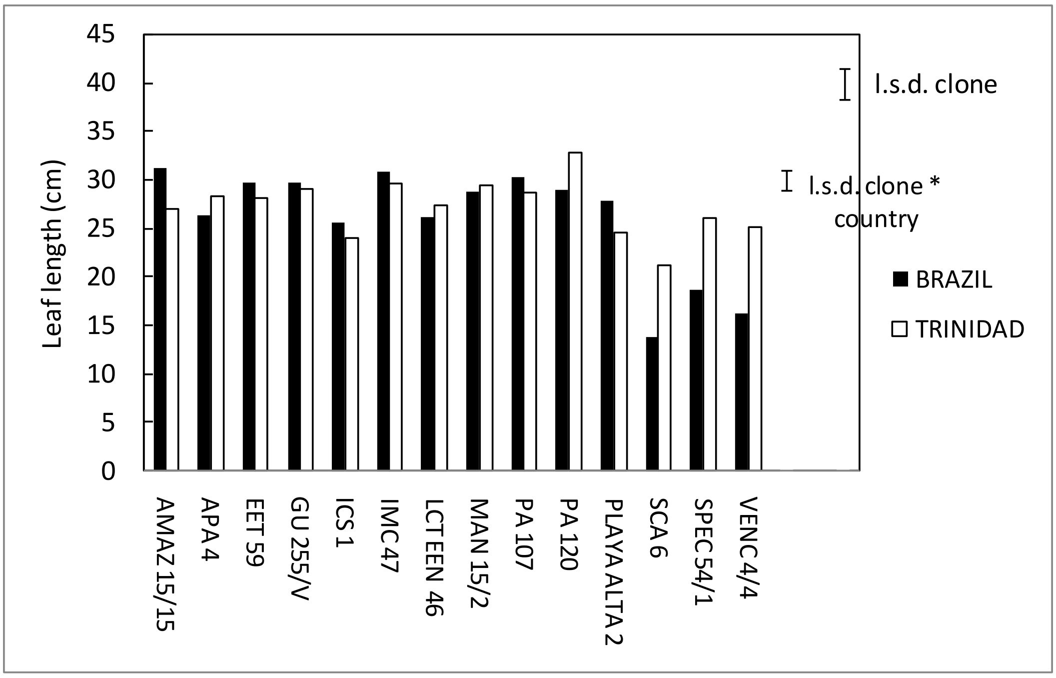

Figure 4: Leaf length of 14 clones in the International Clone Trial in Brazil and Trinidad.

Flushing

Flushing data were recorded monthly over the course of a year in Brazil, Venezuela, Trinidad and Côte d'Ivoire. Analyses were conducted within each country to test for the effect of clone and time of year (month). In all four countries there were significant differences between clones (P<0.001 in all cases), time of year (P<0.001 in all cases) and the interaction between clone and time of year (P<0.001 in all cases). The latter observation may indicate that some genotypes respond to a greater extent than other to environmental stimuli over the course of a year. The seasonal flushing pattern of five clones is illustrated in Figure 5.

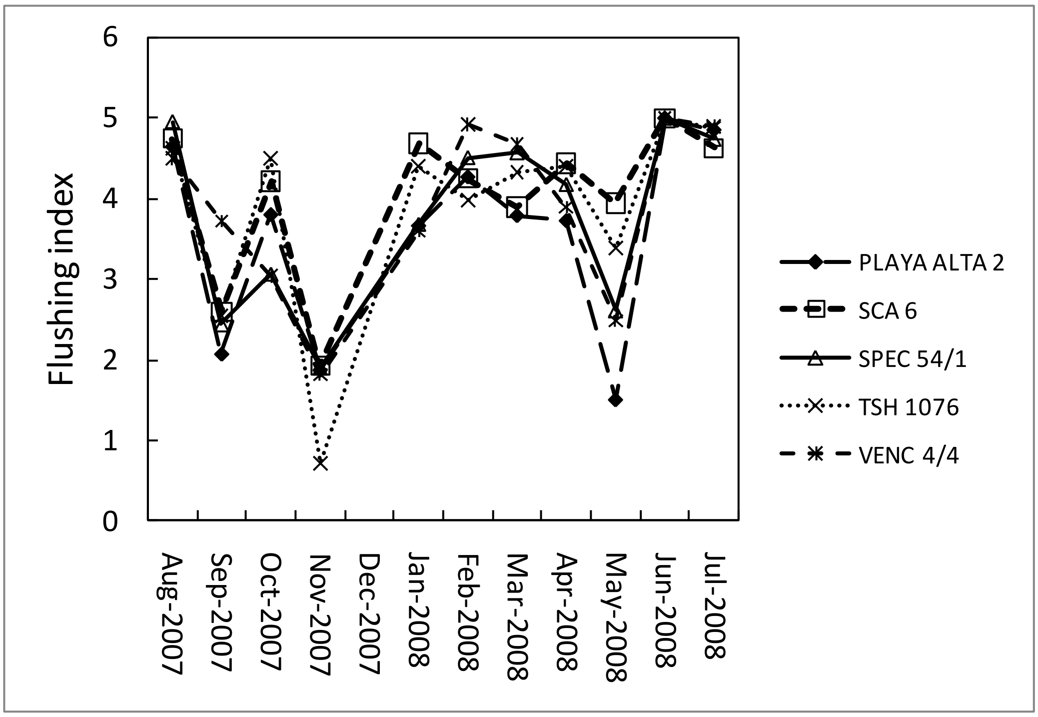

Figure 5: An example of the seasonal variation in flushing intensity of six clones in Trinidad from August 2007 to July 2008

Flowering

Flowering intensity was recorded monthly over the course of a year in Brazil, Venezuela, Trinidad and Côte d'Ivoire. Analyses were conducted within each country to test for the effect of clone and time of year (month). In all four countries there were significant differences between clones ($P<0.001$ in all cases) and time of year ($P<0.001$ in all cases). Furthermore, the interaction between clone and time of year was also significant ($P<0.001$ in all cases) reflecting that particular genotypes show greater seasonal variability in their flower numbers compared with others. An example of the flowering pattern of five clones over the course of a year in Brazil is given in Figure 6. When looking at factors underlying seasonal variation in flowering within the ICT in Brazil, temperature was found to be highly correlated with flowering intensity (flower numbers were suppressed at cooler temperatures). A comparison of regression revealed differences amongst clones in their response to temperature (see Figure 7 for an example of two clones). At the Côte d'Ivoire location a positive correlation was found between rainfall and flowering intensity (Figure 8).

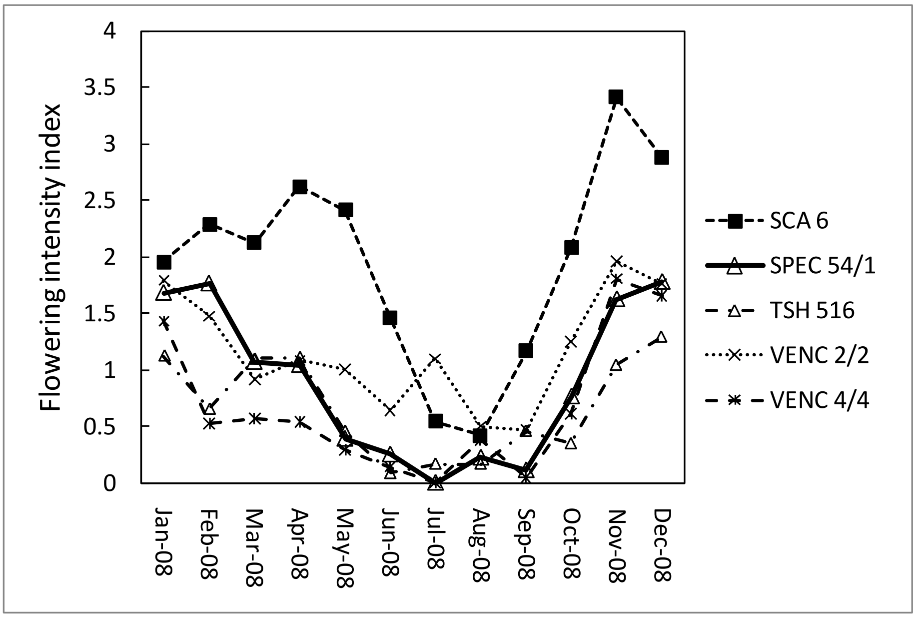

Figure 6: An example of the seasonal variation in flowering intensity of six clones in Brazil between January and December 2008

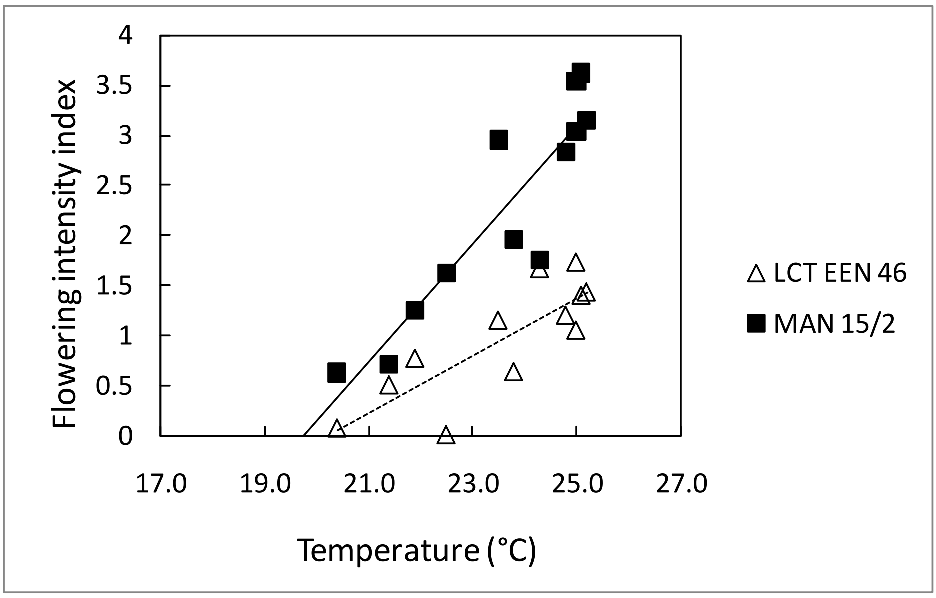

Figure 7: The response of flowering intensity of two contrasting clones to monthly mean temperature in Bahia, Brazil

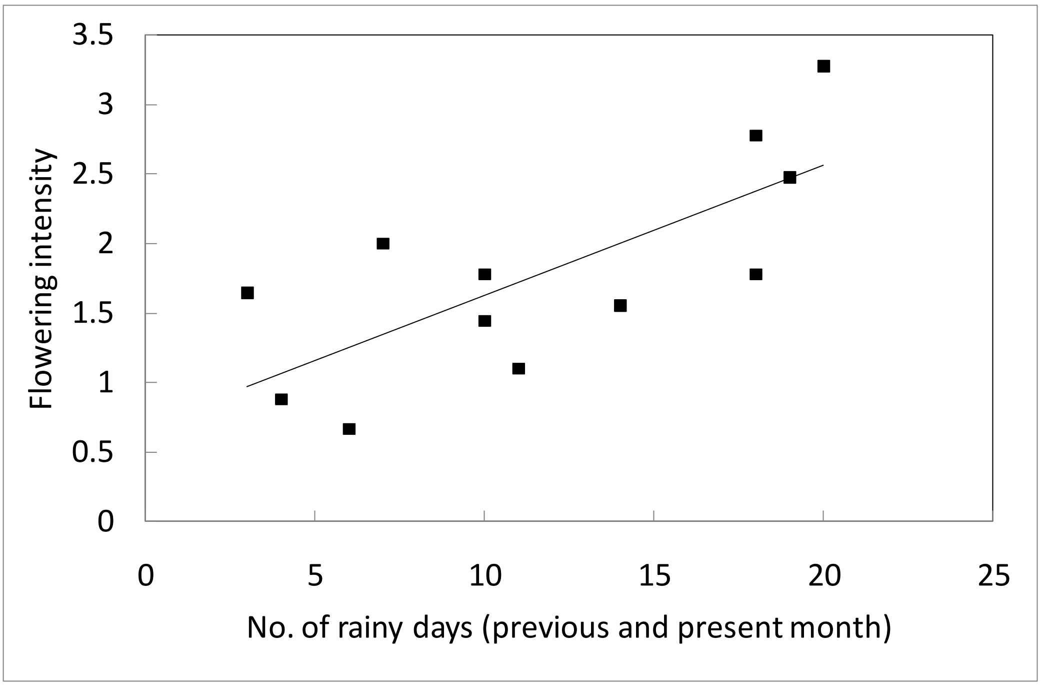

Figure 8: Relationship between rainfall (number of rainy days over two months) and flowering intensity in the International Clone Trial, CNRA, Côte d'Ivoire.

Discussion and Conclusions

The results demonstrate a high degree of genotypic variation in canopy shape and the amount of light transmitted through the canopy confirming previous studies showing genotypic differences in canopy architecture (Yapp and Hadley, 1994; Daymond *et al*., 2002). A unique aspect of the International Clone Trial is that for the first time the performance of a set of clones has been compared in different cocoa growing countries allowing the stability of traits to be assessed. It is implicit in the results that some clones show greater plasticity than others in relation to individual leaf and flush size, which in turn are factors that impact on the amount of light intercepted by the canopy. This has a potential impact on the management of cocoa material in different locations. For example, light interception by the canopy is a factor that influences optimal planting density; if the canopy architecture of particular clones is differentially affected by local environmental conditions then this may in turn impact on what planting density is most appropriate.

The data collected on flushing over the course of a year at four different locations demonstrate genotypic differences in flushing intensity and hence the rate of canopy expansion. Flushing is stimulated by environmental factors such as temperature and water availability (Alvim, 1977). The fact that the magnitude of differences in flushing (and hence the rate of canopy expansion) between clones varied over time in each of the locations suggests that some clones may be more sensitive than others to such stimuli. Experiments under greenhouse conditions using selected clones from the International Clone Trial have also shown that the rate of canopy expansion of different clones varies in response to temperature (Daymond and Hadley, 2008).

Flowering intensity varied across the season in four different locations to a greater extent for some genotypes than others and this is indicative of both genotypic differences in flower numbers but also may indicate differential sensitivity of genotypes to environmental stimuli.

The latter hypothesis is strengthened by the analyses of the data collected at CEPEC, Brazil where differential responses to temperature were observed. Flowering is also known to be affected by other factors including temperature and light intensity (Sale, 1970; Hurd and Cunningham, 1961). The implication of the results is that clones that are more sensitive to particular environmental stresses will show greater variability in their cropping pattern in those areas where such stresses are prevalent.

Overall, it may be concluded that:-
1. Some clones showed greater variability than others both within and across sites with respect to physiological and phenological parameters that impact on yield potential and cropping patterns. The results therefore highlight the need for local evaluation of material in selection and breeding trials.
2. The recommended husbandry practices associated with a given clone (*e.g*. optimal planting and pruning practices) may vary between growing regions.

Acknowledgements

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